BRIEF COMMUNICATIONS

Evolution, 54(4), 2000, pp. 1433–1438

HERITABLE VARIATION IN A FAMILY-DIAGNOSTIC TRAIT

KEITH KAROLY[1] AND JEFFREY K. CONNER[2]
[1]*Biology Department, Reed College, 3203 S.E. Woodstock Boulevard, Portland, Oregon 97202-8199*
E-mail: kkaroly@reed.edu
[2]*Kellogg Biological Station, Department of Botany and Plant Pathology, Michigan State University, 3700 East Gull Lake Drive, Hickory Corners, Michigan 49060*
E-mail: conner@kbs.msu.edu

Abstract.—Derived characters that have not changed during the diversification of a clade provide traits that are diagnostic at higher taxonomic levels. The tetradynamous stamen condition (four long and two short stamens) of the Brassicaceae is an example of a diagnostic trait that has not changed during the diversification of this large flowering plant family. We investigated one hypothesis that might explain the long-term stasis of this trait—that tetradynamous stamens have persisted because of an absence of genetic variation underlying the trait. Through a sib-analysis with *Raphanus raphanistrum* and an artificial selection experiment with *Brassica rapa,* we demonstrate that significant genetic variation is present for the tetradynamous condition in both species and that the trait is therefore not constrained from evolutionary change by a lack of heritable genetic variation.

Key words.—Artificial selection, Brassicaceae, *Brassica rapa,* floral evolution, genetic constraints, genetic variation, *Raphanus raphanistrum.*

Received December 23, 1998. Accepted February 9, 2000.

Characters that distinguish taxa at the level of family or order are likely to have originated with the clade's common ancestor and remained static during the subsequent divergence of species within the taxonomic group. The processes that govern the evolution of these traits that are diagnostic for higher taxa of flowering plants have received little empirical investigation. Although developmental homeostasis may contribute to the relative invariance of such traits within a species (Fenster and Galloway 1997; Cresswell 1998), evolutionary explanations that might account for the stasis across species demonstrated by diagnostic characters include either stabilizing selection or some form of evolutionary constraint. Potential constraints include an absence of genetic variation underlying the character or genetic correlations with traits that are themselves stabilized by selection (Maynard Smith et al. 1985). We have investigated the heritability of a floral trait that is diagnostic for the family Brassicaceae to determine if an absence of genetic variation might constrain the evolution of this taxonomically informative character. Our results for two species of mustard show that heritable variation is present for the trait, indicating that other hypotheses must be invoked to account for the trait's evolutionary stasis.

The majority of the 3000 species in the mustard clade (family Brassicaceae or Cruciferae) possess flowers in which there are four medial stamens that are long and two lateral stamens that are short (the ''tetradynamous'' condition); this character state is considered diagnostic for the family (Cronquist 1981; Endress 1992; Heywood 1993; Zomlefer 1994). The possession of dimorphic stamen lengths cuts across the diversity of mating systems and pollination modes shown by mustard taxa, which range from autogamously selfing to obligately outcrossing, self-incompatible species (Preston 1986). Although the adaptive nature of specific floral traits has long been investigated by evolutionary biologists (reviewed in Stebbins 1974; Lloyd and Barrett 1996), there is no obvious adaptive explanation for the presence of the tetradynamous stamen condition in members of the mustard family.

We have collected genetic data for two self-incompatible species of mustards to directly test the hypothesis that a lack of genetic variation for stamen dimorphism might account for persistence of the tetradynamous condition. Through a half-sib crossing design with *Raphanus raphanistrum* and an artificial selection experiment with *Brassica rapa,* we have determined that stamen dimorphism is heritable and can evolve and that a lack of genetic variation does not constrain the evolution of this taxonomically informative trait.

MATERIALS AND METHODS

Our data for *R. raphanistrum* come from an investigation of the genetic architecture of floral traits reported in Conner and Via (1993). Data were gathered and analyzed for the filament lengths of short and long stamens, but the genetic basis of the stamen length dimorphism was not investigated in the original study (anthers of long and short stamens are approximately the same length and filament length differences account for the tetradynamous condition). The experiment involved a half-sib crossing design that was undertaken with greenhouse plants grown from seed collected from 350 plants in a field population. Fifty sires were crossed with six dams per sire, providing 300 full-sib families. Four offspring per dam were planted in a greenhouse. The length of short and long filaments and petal and pistil dimensions were measured on the third flower to open on 1133 plants. Narrow-sense heritabilities were calculated using the phenotypic variance as the denominator and four times the sire variance component as the numerator (Conner and Via 1993). The genetic coefficient of variation (CV_A) was calculated according to Houle (1992).

Our data for *B. rapa* come from an investigation undertaken by students in Reed College's vascular plant diversity course

TABLE 1. Filament length means and relative filament dimorphism (RFD) for the base populations, the parents of the selected and control lines, and the progeny from all replicates and generations for the *Brassica rapa* artificial selection experiment. Numbers in parentheses are the standard error of the mean. *F*-values are from a one-way ANOVA testing the null hypothesis that F_1 selected and control line mean RFD do not differ. The parents are the 10 plants chosen from the base population for breeding the F_1 of each line, and for the F_2 and F_3 generations (replicate 3) the parents are the 10 plants chosen for breeding from the preceding generation (F_1 and F_2, respectively).

			Parents		Progeny	
Replicate	Trait	Base population	Selected line	Control line	Selected line	Control line
1	plants measured	$n = 100$	$n = 10$	$n = 10$	$n = 20$	$n = 20$
	short filament (mm)	3.07 (0.05)	3.74 (0.17)	3.04 (0.16)	3.01 (0.18)	2.81 (0.16)
	long filament (mm)	5.02 (0.05)	5.10 (0.19)	4.90 (0.21)	4.78 (0.20)	4.91 (0.21)
	RFD	0.612 (0.007)	0.730 (0.009)	0.615 (0.014)	0.617 (0.017)	0.570 (0.012)
					($F = 4.99$, $P = 0.03$)	
2	plants measured	$n = 100$	$n = 10$	$n = 10$	$n = 20$	$n = 20$
	short filament (mm)	2.77 (0.06)	3.41 (0.19)	2.64 (0.10)	2.21 (0.10)	2.22 (0.08)
	long filament (mm)	4.93 (0.07)	5.08 (0.22)	4.59 (0.19)	4.06 (0.11)	4.12 (0.11)
	RFD	0.556 (0.007)	0.669 (0.014)	0.567 (0.009)	0.542 (0.018)	0.537 (0.011)
					($F = 0.06$, $P = 0.81$)	
3	plants measured	$n = 100$	$n = 10$	$n = 10$	$n = 93$	$n = 94$
	short filament (mm)	3.80 (0.07)	4.94 (0.18)	3.74 (0.14)	3.48 (0.08)	2.74 (0.07)
	long filament (mm)	5.91 (0.06)	6.22 (0.18)	5.99 (0.12)	5.16 (0.09)	4.75 (0.09)
	RFD	0.642 (0.009)	0.792 (0.011)	0.624 (0.014)	0.674 (0.009)	0.574 (0.009)
					($F = 57.4$, $P < 0.0001$)	
3	plants measured	F_2 generation	$n = 10$	$n = 10$	$n = 92$	$n = 91$
	short filament (mm)		3.93 (0.13)	2.54 (0.17)	3.39 (0.07)	2.48 (0.06)
	long filament (mm)		5.16 (0.23)	4.34 (0.26)	5.10 (0.06)	4.63 (0.06)
	RFD		0.773 (0.029)	0.581 (0.019)	0.664 (0.011)	0.534 (0.009)
					($F = 83.1$, $P < 0.0001$)	
3	plants measured	F_3 generation	$n = 10$	$n = 10$	$n = 99$	$n = 99$
	short filament (mm)		4.01 (0.29)	2.41 (0.11)	4.65 (0.06)	3.02 (0.06)
	long filament (mm)		4.71 (0.28)	4.62 (0.17)	5.74 (0.05)	5.16 (0.05)
	RFD		0.846 (0.021)	0.524 (0.019)	0.811 (0.010)	0.584 (0.009)
					($F = 300$, $P < 0.0001$)	

(BIO 332). During the past four years, the students have artificially selected for reduced stamen dimorphism, using rapid-cycling stocks of *B. rapa* (Crucifer Genetics Cooperative, University of Wisconsin, Madison; Williams and Hill 1986). These stocks have themselves resulted from nearly 25 years of artificial selection, with their short generation time ($\sim$ 40 days) the outcome of selection on early flowering.

Plants were grown in the Reed College greenhouse in standard potting mix (Sunshine #4) in 3-inch^2 press fit pots (Landmark Plastic) during the winter months of each year (January–March). All plants were watered daily and fertilized weekly (Peat-lite, 200 ppm nitrogen). Temperature and lighting conditions (supplemented with fluorescent or sodium-vapor fixtures) were held roughly constant across generations, but were always identical for the selected and control lines within a single generation. Students were not told the line of origin for any plant they measured during the experiment, and measurement order was completely randomized with the selected and control line plants intermixed. Variation among students in their measuring technique will increase the sampling error in the data, making it more difficult to detect heritable variation in stamen dimorphism. Our results from the *B. rapa* selection experiments should therefore be considered as a conservative assay for genetic variation.

The first year included two replicates of a single generation of artificial selection. The two replicates were carried out independently, involving two separate groups of students. Each student group measured stamen dimorphism in a base population and in the F_1 progeny of separate selected and control lines. In the second year, a single replicate of the selection experiment (including a control line) was initiated by a new group of students (replicate 3 of Table 1) and continued for three generations (base through F_3). Each of the three replicates began with a base population of 100 flowering plants. The lengths of two long and two short filaments were measured using an ocular micrometer on a dissecting microscope. Four flowers were measured for each plant in the base population, with flowers randomly assigned to students to minimize any measurement bias and to ensure that replicate flowers on a plant were measured by different students. Filament length was measured from the point of attachment of the filament to the base of the anther. The relative filament dimorphism (RFD = average short filament length divided by average long filament length) was then calculated for each flower.

Each plant's mean relative filament dimorphism was calculated and used to identify the 10 plants with the *least* dimorphism (i.e., highest RFD), and these 10 plants were then hand-pollinated in all possible combinations to produce seeds of the F_1 of the selected line. Ten additional plants were randomly chosen from the remainder of the base population, and these plants were crossed in all combinations to produce the F_1 of the control line. The first year included two independent replicates of each of the base and F_1 selected and control lines. Seeds for the F_1 were planted and grown to flowering, at which time the students measured the relative filament dimorphism of three flowers per plant for plants of the F_1 generation in both the selected and the control lines (sample sizes are given in Table 1).

In the second year, a single replicate of the experiment

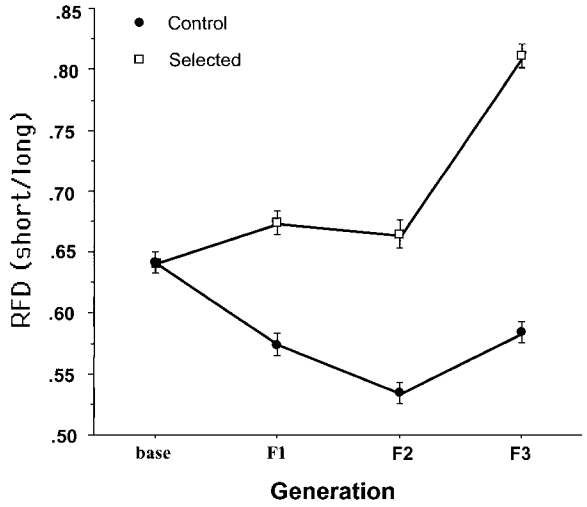

FIG. 1. Mean relative filament dimorphism (RFD: short filament length/long filament length) for the base population and three generations of the selected and control line plants in the third replicate of the *Brassica rapa* selection experiment. Error bars represent +/− one standard error of the mean.

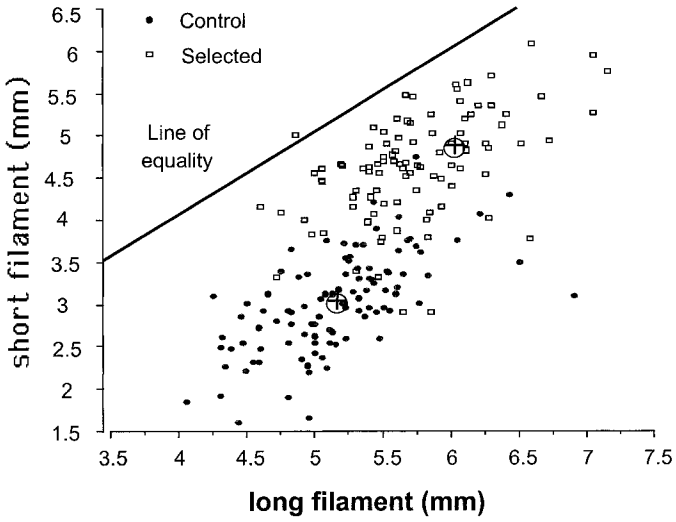

FIG. 2. Bivariate plot showing phenotypic correlations between short and long filament lengths for the selected line and control line of the F_3 generation of the *Brassica rapa* selection experiment (third replicate; $n = 99$ for each line). Bivariate means for each line are indicated by a ⊕. The line of equality is an isocline where RFD = 1.0.

was started with a base and F_1 selected and control lines as described above. Following measurement of the F_1 lines, the 10 plants of the selected line F_1 that had the highest RFD were chosen as parents and crossed in all combinations to provide seeds for the F_2 of the selected line. Ten plants were randomly chosen from the control line F_1 and crossed in all combinations to provide seeds for the F_2 of the control line. The F_2 progeny were measured by students in the third year ($n = 92$ selected line; $n = 91$ control line), and parents were chosen from among these F_2 progeny as described above to provide seed for the F_3 generation. The F_3 generation of the selected and control lines were measured by students in the fourth year ($n = 99$ progeny in each line).

In each of the three replicates, students who measured the base population also measured the F_1 progeny of the control and selected lines. Within the F_2 and the F_3 generations, measurements of control and selected line plants were collected by the same students. Although student identity varied throughout the four years of the study, each of the statistical comparisons we make involves data collected by a single set of students. Statistical analyses were conducted separately for the three replicates, with individual plants used as the units of observation for all analyses. The RFD means for the three base populations were compared using a one-way analysis of variance (ANOVA). The selected and control line means were compared for each progeny generation (F_1 of each replicate and F_2 and F_3 of the third replicate) using a one-way ANOVA. To determine whether the artificial selection altered correlations, the phenotypic correlation between short and long filament lengths was calculated separately for the F_3 progeny generation in the selected and control lines of the third replicate, and these two correlations were statistically compared using a test of homogeneity (Sokal and Rohlf 1981).

RESULTS

The previous analysis of short and long filament lengths in *R. raphanistrum* detected significant additive genetic variation for both traits, with narrow-sense heritability estimates of $h^2 = 0.52$ and $h^2 = 0.58$, respectively (Conner and Via 1993). A statistically significant, positive genetic correlation between the two traits was also reported ($r_G = 0.91$, SE = 0.04; Conner and Via 1993). The average short and long filament lengths for the parent generation were 9.74 mm and 11.70 mm, respectively, giving an average RFD of 0.83. In the current analysis, significant additive genetic variation was found for the ratio of short to long filament lengths (RFD; $F = 2.16$, $P < 0.0001$), with a narrow-sense heritability for the trait estimated as $h^2 = 0.25$ and a $CV_A = 2.37$.

The mean RFD measured in the base populations for the three replicates of the *B. rapa* selection experiment ranged from 0.56 to 0.64, and the differences among replicates were significant (Table 1; one-way ANOVA, $F = 33.0$, $P < 0.0001$). The RFD of the selected line F_1 was greater than the control line RFD for each replicate, although only in the first and third replicate was this difference statistically significant (Table 1). In the third experimental replicate, the selected and control lines continued to diverge for RFD in the F_2 and F_3 generations, with a significant difference between the control and selected lines observed in both generations (Fig. 1, Table 1). For the third replicate, the selected line mean RFD was 118% of the control line mean in the F_1, 125% in the F_2, and 139% in the F_3 generation. By the F_3 generation, several plants with RFD values near 1.0 were found in the selected line (but not control line, Fig. 2). Both the short and long filament lengths in the third replicate were greater in the selected line F_1 than in the control line, but the length difference for the short filaments was proportionally greater (27%) than that for the long filaments (9%), leading to an increased RFD in the selected line. The lengths of

both short and long filaments remained greater in the selected line of the F_2 and F_3 generations relative to the control, with the selected short stamens continuing to show the larger increase (37% and 54%, respectively; Table 1, Fig. 2).

Short and long filament lengths for *B. rapa* showed a strong, positive phenotypic correlation in all three replicates (Fig. 2 for F_3 generation, data not shown for F_1 and F_2 generations). The correlations were significant for both the selected and control line F_3 generation ($r = 0.52$ and $r = 0.64$, respectively; $P < 0.0001$ for both, Fig. 2), but these correlations are not significantly different from each other ($\chi^2 = 1.41$, df = 1).

Discussion

Our results allow us to reject the hypothesis that the tetradynamous stamen trait has persisted in the Brassicaceae because of a lack of genetic variation underlying this trait. For *R. raphanistrum,* significant additive genetic variation was detected for the trait through a controlled breeding experiment, with an estimated narrow-sense heritability of 0.29. For *B. rapa,* a single round of artificial selection on the relative lengths of short and long filaments produced a significant divergence in the trait between selected and control lines in two of three replicate experiments. Continued selection through a second and third round led to increasing divergence between the selected and control lines, demonstrating that the trait can evolve. A strong, positive genetic correlation was reported between short and long filament lengths in *R. raphanistrum* (Conner and Via 1993). A strong and positive phenotypic correlation between these traits has also been observed throughout the replicated experiments with *B. rapa.* Such a positive correlation would be expected to influence any evolutionary response to selection on the filament dimorphism, because selection favoring longer short stamens might lead to longer long stamens as well. Indeed, the divergence observed across generations in the third replicate of the *B. rapa* artificial selection experiment appears to be happening in spite of a positive genetic correlation between the two stamen lengths; although the long stamens are lengthening in the selected line, the short stamens are lengthening to a greater degree relative to controls, and thus the net effect is an increased ratio of short to long stamen lengths (RFD) in the selected lines.

Environmental effects also appear to influence the phenotypic expression of the stamen dimorphism. For *B. rapa,* the RFD was seen to vary significantly among the three replicate base populations, which were derived from the same rapid-cycling stock. Changes observed in the control line mean RFD from the base through F_3 generations of the third replicate may also represent phenotypic responses to a varying environment. Differences in the conditions of greenhouse cultivation are likely to have contributed to this variation between base populations and between generations, because aspects of lighting and temperature were not fully standardized among plantings. Results from a pilot study investigating temperature effects using plants from the selected and control line F_3 generation showed that filament dimorphism varied when the same families were grown in different temperature environments (K. Karoly, unpubl. data). Because the base populations in the three replicates were measured by different people, among-observer variation may have also contributed to the observed differences in the filament dimorphism. Environmental effects and among-observer variation could not have contributed to the divergence in RFD between selected and control lines, however, because the plants being compared were always grown simultaneously, intermixed in the greenhouse, and measured by the same people.

The tetradynamous condition with two short lateral-stamens and four long medial-stamens is nearly universal for species throughout the family Brassicaceae, despite variation in the underlying developmental pattern of stamen initiation (Erbar and Liens 1997). Exceptions are reported in the genera *Romanschulzia, Warea,* and *Stanleya,* where stamens are nearly equal in length (and exserted from the flower; Rollins 1993). These genera are placed by some taxonomists in the tribe Thelypodieae, which is considered to be basal in the family and linked to the closely related family Capparidaceae (Takhtajan 1997), suggesting the possibility that the tetradynamous condition is derived within the mustard family. Other taxonomists have disputed placement of these genera in basal positions (Cronquist 1981), and recent phylogenetic analyses using molecular sequence data placed *Stanleya* in a more derived position within the family (Price et al. 1994; Galloway et al. 1998). The relative degree of filament dimorphism does vary quantitatively among mustard taxa, as shown by the difference in mean RFD for *R. raphanistrum* and *B. rapa* in the current study. We have observed a range in RFD from 0.44 to 0.84 in species from nine genera in the Brassicaceae (*Arabis, Barbarea, Capsella, Iberis, Hesperis, Lobularia, Matthiola, Rorippa,* and two other species of *Brassica*), with a mean RFD of 0.69 (J. K. Conner, unpubl. data).

Given that a lack of genetic variation does not constrain the evolution of tetradynamous stamens in mustards, the reason(s) for the persistence of the trait are unclear. The selective consequences of stamen length differences have been well studied in heterostylous species, but for those taxa there are corresponding style length polymorphisms that are coupled with the stamen height polymorphism (reviewed in Barrett 1992). It is difficult to imagine how stabilizing selection might play a role in the maintenance of tetradynamous stamens in mustards, because there is no obvious adaptive benefit for the stamen dimorphism. Indeed, the dimorphism might be expected to be disadvantageous for successful pollen donation for many species. Both of our study species are self-incompatible and therefore obligately outcrossing. In *R. raphanistrum,* the short filament anthers produce more pollen and have been found to contribute relatively less of their pollen to pollinators when compared to the long filament anthers (Conner et al. 1995; low-level anthers also transfer pollen less efficiently in heterostylous species; Stone and Thomson 1994). The tetradynamous condition would appear to be particularly maladaptive for selfing mustards, where the positioning of short anthers below the stigmatic surface would be expected to limit successful reproduction. Not surprisingly, the short lateral-stamens are reported to be lost sometimes in natural populations of selfing species of mustards (Müller 1961, cited in Bowman and Smyth 1998; J. K. Conner, unpubl. data).

Genetic correlations between the tetradynamous condition

and selectively beneficial traits might offer an alternative explanation for the persistence of the stamen dimorphism. Stamen and petal traits are known to be genetically coupled for many plant taxa, and significant genetic correlations between petal length and the lengths of both short and long filaments were reported for *R. raphanistrum* (Conner and Via 1993). However, no significant genetic correlations occurred between RFD and 10 other traits in *R. raphanistrum* (J. K. Conner, unpubl. data). Comparative studies have suggested that there may be stabilizing selection for some mustards on the position of the long filament anthers relative to the position of the petals (Conner and Sterling 1995). If reduction of the dimorphism requires changes in long stamen length (as observed in our *B. rapa* experiment), then stabilizing selection on long anther position might constrain loss of the dimorphism.

Developmental explanations may also account for the persistence of the tetradynamous condition in mustards. Although we have demonstrated the opportunity for short-term change based on genetic variation at loci affecting both short and long stamen lengths, the long-term stasis of the filament dimorphism could result from an absence of genetic variation at regulatory loci that may have been responsible for the origin of the stamen dimorphism. Developmental homeostasis may also govern the relative constancy of the tetradynamous condition, in which case major changes in the filament dimorphism might only occur as a result of modification of floral developmental homeostasis, which would itself be disadvantageous (Huether 1968; Fenster and Galloway 1997). Although our *B. rapa* selection experiment was designed only to test for the presence of genetic variation, students did note in the F*3* generation that some of the flowers displayed unusual features in their stamens and pistil. Although the frequency of these abnormal features was more common for plants in the selected line when compared to the control line, the difference was not statistically significant.

There are few studies that have experimentally investigated the role played by natural selection or genetic constraints in the evolution of traits that are diagnostic for higher order plant taxa. Holtorp (1944) carried out artificial selection on several species of dicots (including mustards) in which three cotyledons were occasionally found in the seedling rather than the normal two. Following several generations of selection for tricotyledony, Holtorp observed an increased frequency of tricotyledons and concluded that the dicotyledon condition was not constrained by a lack of genetic variation. Variation in pappus number from the pentamerous condition has been shown to be heritable in the composite genus *Microseris* (Asteraceae; Vlot and Bachmann 1991; Vlot et al. 1992). Huether (1968) was able to demonstrate through artificial selection that variability in corolla lobe number in *Linanthus androsaceus* (Polemoniaceae) has a genetic basis. The normal five-lobed condition that typifies members of the phlox family was significantly more variable after only five generations of artificial selection favoring decreased canalization. Although Huether (1969) concluded that the constancy of five lobes in the Polemoniaceae resulted from natural selection, Stebbins (1974) reported that pollinators of *L. androsaceus* in the field did not discriminate between normal five-lobed and naturally occurring six-lobed or four-lobed flowers. Traits that are diagnostic of higher order taxa provide some of the best opportunities to study evolutionary processes that may be responsible for long-term evolutionary stasis and change, and we believe such traits are worthy of much greater attention.

Acknowledgments

We thank the students of the vascular plant diversity course at Reed College for their assistance with data collection, and M. Mayfield and S. Bowman for greenhouse assistance and help with the *Brassica rapa* crosses. We also thank P. Wilson, J. Kohn, C. Goodwillie, C. Fenster, J. Brunet, A. Sakai, S. Andersson, M. Dudash, and three anonymous reviewers for helpful comments on the research and the manuscript. This research was supported by National Institutes of Health NRSA grant GM 13066 and National Science Foundation grant DEB 93–18388 to JC. This is KBS contribution no. 900.

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Corresponding Editor: M. Dudash

Evolution, 54(4), 2000, pp. 1438–1444

HABITAT-RELATED ADAPTIVE PROPERTIES OF PLANT CUTICULAR LIPIDS

RICHARD S. DODD[1] AND ZARA AFZAL-RAFII[2]
[1]*Environmental Science Policy and Management, University of California at Berkeley, 145 Mulford Hall, Berkeley, California 94720*
E-mail dodd@nature.berkeley.edu
[2]*Institut Méditerranéen d'Ecologie et Paléoécologie, UA 1152, Université d'Aix-Marseille III, 13397 Marseille, France*
E-mail chain.rafii@botmed.u-3mrs.fr

Abstract.—Cuticular permeability is thought to be related to the biophysical properties of cuticular waxes and, in turn, to the chemical composition of this boundary layer. However, thus far evidence for this relationship has been elusive. We investigated possible correlations between habitat and inter- and intraspecific variations in two parameters likeley to affect crystallinity and transition melting temperatures of waxes. Three species from the family Cupressaceae (*Austrocedrus chilensis, Fitzroya cupressoides, Pilgerodendron uviferm*) were selected as a model system because they are closely related and form a continuum over 20° of latitude in South America that includes important climatic differences. We found major divergence among the three species and more fine-scale population differentiation for *A. chilensis* and *P. uviferum* in weighted mean carbon number (*N*) and dispersion about this mean (*d*). Broad-sense heritabilities, estimated from ramet-ortet regressions and from analysis of variance among ortets of *F. cupressoides* were 0.92 for *N* and from 0.64–0.76 for *d*. Even in areas of close sympatry, species maintained their unique biochemical characteristics, thus supporting the genetic basis of cuticular hydrocarbons. Both species and population patterns suggest that natural selection has favored cuticular hydrocarbon mixes that provide differential fitness in the face of habitat differences in water stress and temperature.

Key words.—Cupressaceae, cuticular waxes, genetic adaptation, heritabilities, hydrocarbons, water stress.

Received August 2, 1999. Accepted January 6, 2000.

The wax coating on cuticles, which provides the interface between transpiring organs (particularly leaves) and the atmosphere, is one of several adaptations that have arisen in the evolution of terrestrial plants that enhances resistance to water loss. This boundary layer is composed of a complex mixture of aliphatic, cyclic, and phenolic constituents (Baker 1982) that covers the nonporous leaf surface. Its importance in regulating the water balance of the plant is indicated by the greatly elevated levels of cuticular permeability that accompany solvent extraction of surface lipids that leaves the cuticle otherwise intact (Schönherr and Reiderer 1989). Under moderate to low water stress, stomata are probably the major route by which moisture is lost from the leaf (Kersteins 1996), but under high water stress, when stomata have closed, evapotranspiration must be through the cuticular membrane.

If cuticular waxes are important in controlling water balance, variations in the effectiveness of this layer should provide differential fitness on which selection can act. Schreiber and Reiderer (1996) have indeed shown that permeabilities of cuticular membranes, when tested under laboratory conditions, vary among species native to widely different climatic zones, suggesting habitat-driven genetic adaptation. Similarly, insect studies have shown environmentally correlated variation in cuticular permeability (Hadley and Schulz 1987), suggesting genetic adaptation in the underlying properties of surface waxes. The genetics of these underlying properties is of major interest in evolutionary biology, but this is not simple to elucidate given the complexity of the mixture of cuticular waxes and our inadequate knowledge of their structural organization. For example, although Schreiber and Reiderer (1996) found differences in membrane permeabilities among plants from different climatic origins, they were unable to correlate permeability with the total amount of cuticular wax or the melting points of the waxes. Insect biologists seem to have had greater success in relating permeability to phase phenomena of waxes (Lockey 1988; Gibbs